Definition and determination of in vitro antibiotic susceptibility breakpoints for bacteria

Jacques Sirot[1], *Patrice Courvalin*[2] *and Claude-James Soussy*[3]

[1]Faculté de Médecine, Service de Bactériologie-Virologie, 28 Place Henri Dunant, 63001 Clermont-Ferrand, France; [2]Centre National de Référence des Antibiotiques, Unité des Agents Antibactériens, CNRS EP J0058, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France and [3]CHU Henri Mondor, Service de Bactériologie, 51 av. de Lattre de Tassigny, 94010 Créteil Cedex, France

With the other members of the 'Comité de l'Antibiogramme de la Société Française de Microbiologie' (CA-SFM) : J.F. Acar, H. Chardon, P. Choutet, H. Dabernat, H. Drugeon, L. Dubreuil, J.P. Flandrois, F. Goldstein, C. Morel, A. Philippon, B. Rouveix, A. Thabaut.

Clinical categorization, the classification of bacterial strains into susceptible, intermediate and resistant categories with regard to an antibiotic, is based on the critical values determined for minimum inhibitory concentrations (MICs) or for inhibition zone diameters. This classification represents the last step in the evaluation of a new antibiotic and should take into account all the information gathered during the appraisal. It is an essential guide for therapy [1–3].

The antibiotic susceptibility (or resistance) of a strain cannot be measured directly but must be deduced from the in vitro activity of the antibiotic. Among the various methods available, MIC determination is the most widely used to assess in vitro activity for clinical categorization of clinical isolates. To convert MIC values into susceptible or resistant categories, i.e., to assess whether it is possible to treat an infection by a given antibiotic, reference is made to the critical values recommended by national committees such as the Comité de l'Antibiogramme of the Société Française de Microbiologie (CA-SFM) in France, the British Society for Antimicrobial Chemotherapy (BSAC), or the National Committee for Clinical Laboratory Standards (NCCLS) in the USA. The values are established on the basis of bacteriologic, pharmacokinetic and clinical criteria [4–6].

The differences between the critical values recommended by the various committees arise from the contrasting definitions of susceptibility and resistance. Two definitions of resistance have been proposed by the World Health Organization (WHO):

1. A bacterial strain is considered resistant when it tolerates a concentration of antibiotic considerably higher than that which inhibits the development of the majority of the other strains of the same species.
2. A bacterial strain is considered resistant when the concentration of antibiotic that it is able to tolerate is markedly higher than the concentration achievable in vivo.

The first definition corresponds to 'categories of bacterial populations' and the second to 'therapeutic categories' on the basis of pharmacokinetic and clinical criteria [7–9].

Despite these differences, it seems that a consensus has been reached, at least in Europe, for the establishment of critical values based on the three criteria analyzed below:

- MIC distribution of bacterial populations belonging to different species and harboring genetically and biochemically characterized resistance mechanisms;
- pharmacokinetics, at usual and maximum dosages, using the different routes of administration;
- correlation between the clinical and bacteriologic results for the therapeutic indications assigned by the different ministries of health.

BACTERIOLOGIC CRITERIA

In the simplest case, there is a bimodal distribution of the MICs for bacterial strains belonging to the same

species that facilitates the characterization of two populations: one with low MICs, evenly distributed; another with much higher MICs corresponding to strains possessing a resistance mechanism. Between these two populations there are few or no strains (Figure 1). However, and quite unfortunately, the distribution is often multimodal because of the multiplicity of resistance mechanisms. In the latter case, a larger number of strains is present between the limits separating the two main populations (Figure 2). Strain populations may even not be well defined (Figure 3). This is, for example, generally the case for enterobacteria with regard to fluoroquinolones, where some of the nalidixic acid-resistant strains appear to belong to the drug-susceptible population. Thus, in this example, approximately 30% of the nalidixic acid-resistant strains are inhibited by concentrations of ofloxacin $\leq$ 1 mg/L and should *a priori* have the same behavior in clinical settings as strains devoid of resistance mechanisms.

PHARMACOKINETICS

With regard to the relationship between the MIC determined in vitro and the antibiotic concentration at the site of infection, a strain is considered susceptible if its MIC is lower than the blood or tissues levels or those in the main pathologic center of infection following usual doses. The strain is considered resistant if the MIC is greater than the highest concentration achievable in vivo. It is considered intermediate if the MIC is higher than blood levels and lower than levels obtained at certain sites of the organism or in certain fluids, in particular the urine and the bile. Thus, the lower breakpoint discriminating the susceptible and intermediate categories is based essentially on antibiotic serum concentrations obtained with usual doses.

As an aid to the determination of provisional critical values prior to clinical studies, pharmacokinetics should provide the following information:

- Absorption: for antibiotics prescribed by the oral route, knowledge of the absolute biodisposibility and the variability in digestive absorption is important.
- Distribution: maximum serum concentration (C_{max}) for a given dosage and route of administration and the time required to reach that peak (T_{max}); rate of elimination, generally assessed by determination of the plasma half-life ($t_{1/2}$); diffusion in the humors and

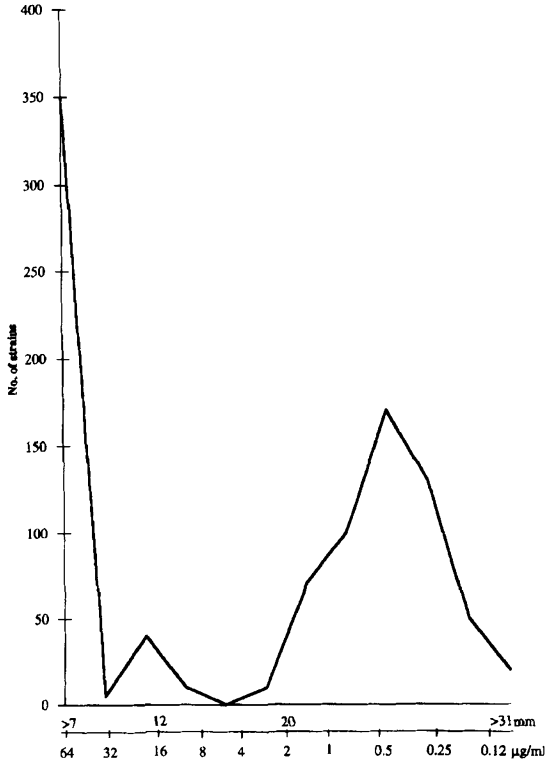

Figure 1 Distribution of tetracycline MICs and of corresponding inhibition zone diameters (30-µg disks) for 1000 strains of *Staphylococcus aureus*.

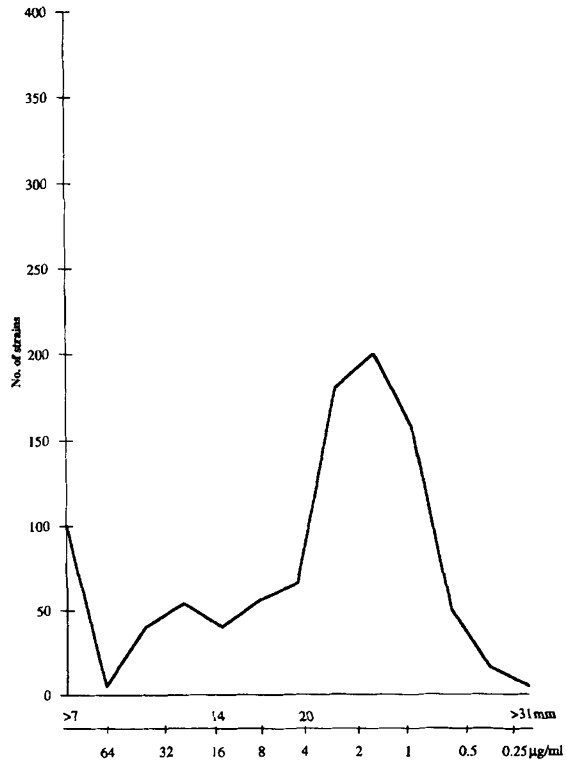

Figure 2 Distribution of gentamicin MICs and of corresponding inhibition zone diameters (30-µg disks) for 1000 strains of *Klebsiella pneumoniae*.

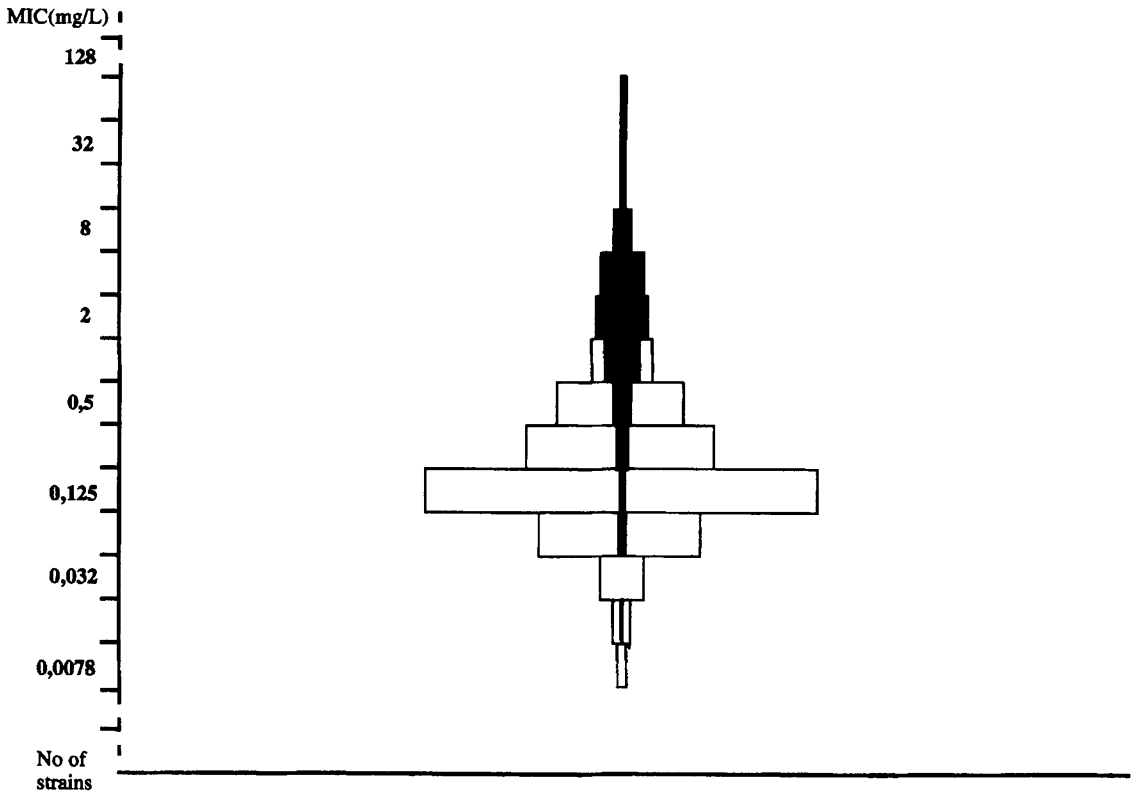

Figure 3 Distribution of ofloxacin MICs for 850 strains of *Enterobacteriaceae*: □, nalidixic acid-susceptible strains; ■, nalidixic acid-resistant strains.

tissues (keeping in mind that the values observed for a given antibiotic and a given tissue often vary a great deal depending on methodology; however, the results obtained can help in defining the clinical studies that have to be undertaken); extent of protein binding.

- Biotransformation: including detailed information on the antibacterial and pharmacokinetic properties of the possible metabolites.
- Elimination routes: urinary, biliary, digestive, giving the percentage of active form.

The level of active drug achieved at the site of infection is a major factor in successful antibiotic therapy. The main difficulty in defining breakpoints, according to this observation, is that pharmacokinetic parameters and active concentrations at infection sites are usually unknown. However, most bacteria are located in extracellular tissue fluids and the penetration of the majority of antibiotics into extracellular fluids results in (free) drug concentrations similar to those in serum at steady state. The pharmacokinetics of drug in serum may therefore represent those in compartments where passive diffusion governs antibiotic penetration [10].

Although the actual relationship between pharmacokinetic parameters and the in vitro MIC has not been established, some formulas taking into account pharmacologic and microbiological factors have been proposed for the calculation of breakpoints [5].

The following parameters related to activity are taken into consideration by the CA-SFM in the determination of breakpoints [11]:

- the peak serum concentration (C_{max}) achieved with the recommended dosage;
- the serum concentration achieved after the distribution phase (the equilibrium is usually reached 1 h after administration);
- the drug half-life ($t_{1/2}$) and the time during which concentration in serum exceeds the MIC;
- a restrictive or semi-restrictive protein binding if >75%.

Following recommended dosage, either peak concentration/MIC ratio above 4 to 8 or the time for

which plasma concentrations exceed the MIC should be taken into account in defining the lower breakpoint for drugs that, respectively, exhibit a concentration-dependent (e.g., aminoglycosides, fluoroquinolones) or a time-dependent killing (e.g., β-lactams, glycopeptides). The higher breakpoint should allow for the maximum serum concentration, the toxicity and the increased antibiotic concentration in certain sites.

CLINICAL CRITERIA

The clinical criteria form a necessary complement to those described above and provide information on the correlation between therapeutic success or failure and the proposed critical concentrations. To put it simply, a resistant strain escapes treatment. It is theoretically possible to know if patients infected by a bacterial strain with an MIC of X mg/L will recover after a usual treatment and if patients infected by a strain with an MIC higher than Y mg/L will not recover. However, the results are usually debatable, since the recovery criteria are not always clearly defined and may differ depending upon the evaluation.

The so-called 'early clinical trial' corresponds to the first therapeutic trial in humans of a new drug. This should include infections in which the causative bacterial agent belongs to the homogeneous population of strains with low MICs, below the low critical concentration proposed. 'Only a few detailed bacteriologic observations are required and may be sufficient to ascertain the clinical activity of a new antibiotic' [14].

It ought to be possible, during the phase III clinical trials, to clarify the clinical results obtained previously and, in particular, to analyze the bacteriologic failures. These can be interpreted correctly only if one takes into account the dosages used, the values of the serum, and, if possible, humoral and tissue concentrations, and the MIC and resistance phenotype of the strain responsible for the infection. Cases of infections due to strains with MICs close to the lower breakpoint should be carefully analyzed. The critical concentrations proposed cannot be considered definitive unless they correlate with the clinical results [12].

Three categories of strains are classically defined:

1. Susceptible strains. Their MICs are attainable by treatment with usual doses by general routes. These MICs are lower than the levels achieved in the blood or tissues or those present in the main pathologic foci.
2. Resistant strains. Their MICs will probably not be attained, irrespective of the treatment, since they are greater than the highest concentrations achievable in vivo.
3. Intermediate strains. Their MICs are attainable only with high doses of antibiotics close to toxicity, or in cases where local treatment is possible or physiologic concentrations occurs at the site of infection.

Thus, the last category includes strains with MICs in the range of the concentrations achievable in vivo, i.e. higher than the blood levels but lower than those obtained in certain sites of the organism. It also includes strains that do not belong to the susceptible population but do not appear clearly resistant; certain strains may express a low-level resistance that could increase under therapy. The intermediate category also represents an imprecise zone, sometimes called the 'buffer zone', which allows for technical and biological uncertainties [13].

Since it is largely based on pharmacokinetics and clinical criteria, the clinical categorization defines more closely the in vitro/in vivo parallelism. The size of the intermediate zone is sufficient to allow interpretation that takes into account concentrations at the infection site, and which is not dependent upon the bacterial species involved. The major defect in clinical categorization is that it relies largely on pharmacokinetic parameters that are sometimes difficult to define. The breakpoints delimiting the categories are therefore inevitably approximations which, while reflecting a real situation, make it difficult to achieve international standardized criteria [14].

RELATIONSHIP BETWEEN MICs AND INHIBITION ZONE DIAMETERS

Determination of critical diameters that delineate the susceptible, intermediate and resistant categories requires, for each antibiotic, a good correlation between the inhibition zone diameters and the corresponding MICs. Generally, 100 to 150 strains representative of the most common clinical isolates with MICs evenly distributed over the range studied are tested simultaneously by agar dilution (MICs) and by disk diffusion (diameters) [15]. The regression line between the MIC values (logarithm to the base 2) on the x-axis and the inhibition diameters (arithmetic scale) on the y-axis indicates the degree of correlation of the distributions observed. The critical diameters can then be deduced from the critical concentrations (Figure 4).

In case of poor correlation, the scattergram obtained with several hundreds of strains collected during a multicenter study provides a mean for determination of the critical diameters. In this analysis, the percentage of major discrepancies, in particular Rs discrepancies (strain considered to be resistant by agar

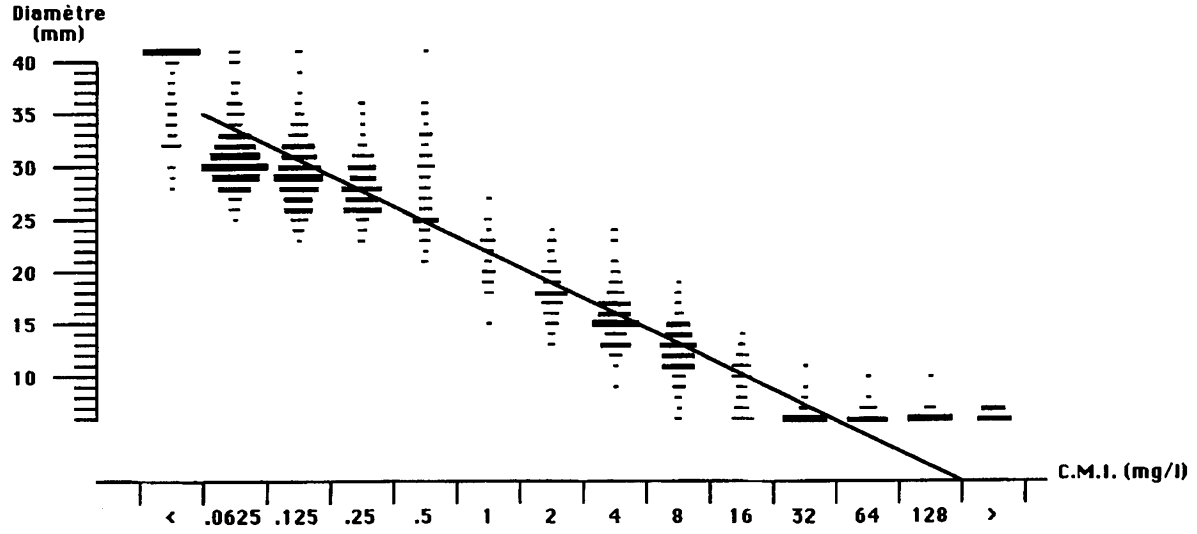

Figure 4 Correlation between the inhibition zone diameters (mm) obtained with a 5-μg fleroxacin disk (*y*-axis) and the $\log_2$ of the MIC (μg/mL) of fleroxacin determined by agar dilution (*x*-axis) for 649 strains. The least-squares line of regression (*x* on *y*) and the linear regression coefficient (*r*) were obtained with a Tektronix computer.

dilution and susceptible by agar diffusion) is kept as low as possible (Figure 5) [16,17].

The disk-agar diffusion test is standardized for bacteria that grow rapidly on usual media. The interpretation criteria should therefore be applied cautiously for slow-growing bacteria or for those requiring supplemented medium or anaerobiosis. Certain resistance mechanisms, in particular due to detoxifying enzymes, are not always detected by the disk test using the standard breakpoints; special study conditions and 'interpretive reading' of the results based on a thorough knowledge of the mechanisms of resistance may be required. Certain significant examples have been reported [18,19].

CONCLUSION

Critical values (breakpoints) for clinical categorization of bacterial strains with regard to a given antibiotic should be proposed at various stages:

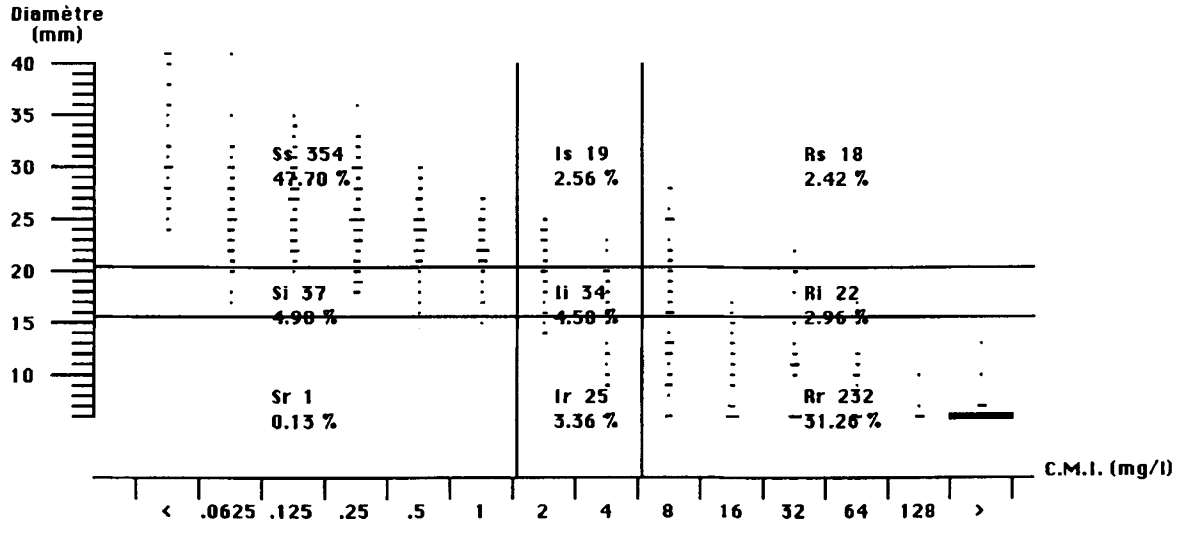

Figure 5 Dirithromycin. Distribution of 547 clinical isolates belonging to different species in susceptible, intermediate and resistant clinical categories according to MICs (S.I.R.) and inhibition zone diameters (s, i, r) obtained with 15-μg disks. Ss, Ii and Rr denote agreement; Is, Si, Ir and Ri, denote minor discrepancies; Sr and Rs denote major discrepancies.

- Before the antibiotic is released on the market, provisional values can be obtained by analysis of the in vitro spectrum of activity, the MIC distribution of bacterial populations and the results of pharmacokinetics.
- More definitive values can only be established once authorization of release on the market has been granted and according to the clinical results obtained, the therapeutic indications and the recommended dosages.
- Subsequent revision of these values may occur in light of the information provided by monitoring bacterial resistance and possible modifications of the therapeutic indications or dosages.

Determination of critical values is always a compromise that can give rise to a certain number of difficulties:

- Strains belonging to a homogeneous population can fall into different classes if, for pharmacokinetic reasons, the low breakpoint divides the population in two. This is one of the main reasons for introducing an intermediate category.
- Strains possessing a weakly expressed resistance mechanism that requires particular detection conditions can be considered as susceptible on the basis of the low breakpoint. Because of risk of clinical failure, decrease of this latter value or interpretive reading of the susceptibility test can ensure classification of the strain in the intermediate or resistant category. This approach will, most probably, be made easier in the future by using artificial intelligence [20].

The MIC and zone diameter breakpoints recommended by the CA-SFM are provided at the end of this issue.

References

1. Acar JF, Goldstein FW. The disc susceptibility test. In Lorian V, ed. Antibiotics in laboratory medicine. Baltimore, London: Williams and Wilkins, 1985: 27–63.
2. Denys GA, Hansen SL, Pope WA, Lilli H, Hejna M. Collaborative investigation of the accuracy and reproducibility of sceptor breakpoint susceptibility panels. J Clin Microbiol 1987; 25: 2189–92.
3. Waterworth PM. Sensitivity testing by the breakpoint method. J Antimicrob Chemother 1981; 7: 117–26.
4. Comité de l'Antibiogramme de la Société Française de Microbiologie. Communiqué 1995. (In insert.) Pathol Biol, 1995; 43(8): I–VIII.
5. British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing. J Antimicrob Chemother 1991; 27 (Supp. D): 1–47.
6. National Committee for Clinical Laboratory Standards. Development of in vitro susceptibility testing criteria and quality control parameters. Tentative guideline. NCLLS Document M23–T. Villanova, Pa.: National Committee for Clinical Laboratory Standards, 1990.
7. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45: 493–6.
8. Daguet GL, Chabbert YA. Techniques en bactériologie. 3. Sérologie bactérienne, antibiotiques en bactériologie médicale. Paris: Flammarion, 1972.
9. Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathol Microbio Scand 1971; Section B, Supplement 217.
10. Lebel M. Tissue and tissue fluid penetration of antibiotics. Antimicrob Newslett 1989; 6: 69–72.
11. Cluzel R. Concentrations critiques du céfixime et charge du disque. Presse Med 1989; 18:1567–8.
12. Mouton Y, Beuscart C, Soussy CJ. Efficacité et tolérance de la pipéracilline chez 333 malades. Presse Med 1986; 15: 2347–50.
13. Sirot J. Catégorie intermédiaire. In Courvalin P, Goldstein F, Philippon A, Sirot J, eds. L'Antibiogramme. Paris: MPC Vigot, 1985: 155–61.
14. Chabbert YA. Sensibilité bactérienne aux antibiotiques. In Le Minor L, Véron M, eds. Bactériologie médicale. Paris: Flammarion, 1982: 204–12.
15. Drugeon HB, Juvin ME, Caillon J, Courtieu AL. Assessment of formulas for calculating critical concentration by the agar diffusion method. Antimicrob Agents Chemother 1987; 31: 870–5.
16. Flandrois JP, Carret G. Approche non paramétrique de la détermination des diamètres critiques. Comparaison avec les méthodes conventionnelles. Pathol Biol 1984; 32: 488–91.
17. Tekaïa F, Derlot E, Claverie JM, Courvalin P. Contrôle de qualité des études multicentriques d'évaluation de l'activité in vitro des antibiotiques. Pathol Biol 1987; 35: 687–91.
18. Courvalin P. Interpretive reading of antimicrobial susceptibility tests. Molecular analysis and therapeutic interpretation of in vitro tests to improve antibiotic therapy. ASM News 1992, 58: 368–75.
19. Goldstein F. Limites de l'antibiogramme. In Courvalin P, Goldstein F, Philippon A, Sirot J, eds. L'Antibiogramme. Paris: MPC Vigot,1985: 19–22.
20. Courvalin P. Perspectives. In Courvalin P, Flandrois JP, Goldstein F, Philippon A, Quentin C, Sirot J, eds. L'Antibiogramme automatisé. Paris: MPC Vigot, 1988: 131–5.